REMARKS

This is in response to the official action dated April 11, 2011 Reconsideration in view of the following is respectfully requested.

Claim Status

Claims 2-6, 13, 14 are pending in the application. Claims 7-12, 15 and 16 stand withdrawn. Claims 2-6, 13 and 14 stand rejected.

Enablement/ 35 USC § 112, 1st para, claims 2-6, 13 & 14

Examiner rejected the claims for leaving out required culture conditions and suggested to amend the limitation directed to the culture condition. Accordingly, the term "without addition of exogenic growth-promoting compounds and without addition of antibiotics and fungistatic agents" has been added to claim 6, which should obviate the examiner's rejection. Support for this term can be found on page 8, item (a), and page 12, paragraph 2. Notably, claims 2-5, 13 and 14 depend from claim 6.

Indefiniteness/35 USC § 112, 2st para – cell culture medium, claims 2, 3, (4)

Examiner rejected claims 2-4 due to a clause directed to alpha-MEM and HAM-F12 medium and requested clarification. This clause is present only in claims 2 and 3, in which it has been corrected, clarifying that "the cell culture medium has a ratio of alpha-MEM medium and HAM-F12 medium which is between 2:1 and 1:2".

Indefiniteness/35 USC § 112, 2st para – humidity, claims 2 & 3

The Examiner requested clarification of the subject matter for the humidity range. Applicant amended the relevant claims (claims 2 and 3) accordingly, clarifying the subject matter as follows: "at 36.8-37°C in air containing 5% carbon dioxide and **said air** having a humidity of 85-95%. (emphasis added).

Indefiniteness/35 USC § 112, 2st para –autologous, claims 2-6, 13, 14

The examiner requested clarification of the term "autologous (serum)", specifically, what the serum is autologous to, cultured cells, or source thereof, or the recipient of the resulting transplant. Typically the serum will be autologous to both, since source and recipient typically are the same person/the patient, though close siblings or matched donors may also be acceptable. Applicant amended claim 6 accordingly, clarifying "autologous serum **autologuous to the source of the cultured cells and/or intended recipient of the transplant.**" Notably, all other relevant claims (2-5, 13, 14) recite claim 6.

Indefiniteness/35 USC § 112, 2st para –internal, claim 13

The unclear term has been deleted from the claim, which should obviate the Examiner's rejection.

Indefiniteness/35 USC § 112, 2st para – claim 5 limitation/3D transplants, claim 13

Applicant incorporates the culture vessel limitations of claim 5 into claim 13.

Unobviousness, 35 USC 103 – Masuda '400 & Okuma

The features of claim 14 (mixture of annulus fibrosus/AF and nucleus pulposus/NP cells) have been incorporated into claim 6. All claims accordingly recite a mixture of AF and NP cells. The Examiner asserts that either NP or AF cells **alone** can be used as chondrogenic cells (and presumably resulting transplants), relying on Masuda '400, in particular paragraph 34. This is not the case, as disclosed in both Masuda '400 and Okuma:

Masuda '400 teaches that to produce the important chondrocyte CM or ECM (cellular matrix and

extra cellular matrix, respectively), significantly more is required, see Masuda '400, paragraphs 41-53.

Monolayer/2D cultivation is believed to be detrimental in the art and to cause cells to lose their phenotype and ability to incorporate major macromolecules into a intervertebral disc tissue matrix, compare Masuda **'473**, paragraph 22. Cells need to be cultured in agarose or alginate to preserve their phenotype (spherical shape and matrix formation), see Masuda '400, paragraph 42, and for amplification of cells, growth medium containing growth factors such as fetal serum or specifically added growth factors are needed to maintain the chondrocyte phenotype. This presumably is one of Masuda '400's "appropriate stimuli" that a cell first has to be exposed to before becoming capable of producing and secreting components characteristic of cartilage tissue, as Masuda '400 discussed in earlier in paragraph 34, which the Examiner cited in isolation.

Only in some of Masuda '400's embodiments do the cells have the ability to synthesize certain matrix components. With the matrix established in cell culture, cells are less likely to become dedifferentiated according to Masuda '400 (paragraph 49). Notably, the culture conditions and in particular physical arrangement of culture system (2D/3D) and presence of various growth factors will alter the CM and ECM (Masuda '400 paragraph 50). While Masuda '400 states in paragraph 34 (which is the only paragraph the Examiner cites), that chondrogenic cells **can** be isolated from a large number of sources such as articular/joint cartilage, costal cartilage, nasal cartilage, etc., etc., and, among many, also intervertebral discs, in the context of Masuda '400's complete disclosure, it becomes apparent that each of these sources will require extensive

modification way beyond routine techniques. This is especially true if the objective is not only the isolation of cells but their further suitability to be reinserted as a transplant.

Accordingly, the applicant believes that if Masuda '400's complete disclosure is taken into account, Masuda '400 does not teach **how** to obtain chondrogenic cells from NP or AF or **how** to culture such cells, nor how to culture cells useful as transplants.

What Masuda '400 does teach is that some chondrogenic cells can be cultured in agarose (to retain their spherical shape and CM formation, para 42), and for enhanced CM production (which in addition to ECM is relevant for resinsertion as transplant) **fetal bovine serum is necessary** (paragraph 42). In some embodiments the medium can contain autologuous serum – in case of all of Masuda '400's examples this would be adult **bovine** serum – Masuda '400's fig./example 1 is **bovine** ARC cartilage tissue, fig./example 2 is **bovine** ARC tissue, and the last figure/example 3, again, is **bovine** ARC tissue. Accordingly there is little guidance in Masuda '400 how to adapt the general teaching to the human situation, given the lack of general availability of fetal human serum, and the significant differences in cell culture method and transplant formation. The little guidance present would lead the skilled person to experiment with "semi-solid" 3D agarose cultures and growth factors like IGF-1 disclosed by Masuda '400, to be attached to a bone or bone-substitute scaffold before transplantation. Masuda '400 does not disclose how to perform a method as claimed. Notably, present claims are directed to a method that employs a mixed culture of AF and NP cells in monolayer. Further, the claimed method does not require exogenic growth-promoting compounds, nor is a scaffold necessarily needed when the resulting cells are reinserted/transplanted.

While the Examiner merely cites usage as chondrogenic cells, this as such is insufficient for forming a successful transplant – apart from suitability in terms of CM and ECM synthesis and proliferation, Masuda '400 uses the suitable cultured cells in combination with a scaffold with "at least one" natural cancellous bone or bone substitute material, compare Masuda '400, paragraph 18. **Okuma** uses a different approach but encounters similar difficulties, compare Okuma, experimental study 2, p.994: NP cells cultured alone did not grow and differed morphologically from cells in their in vivo environment, e.g. they were polygonal rather than spherical. For the advantageous effect in reinsertion of the transplant NP cells on their own will not work, see under "Discussion" in Masuda '400, p.996, left column, end of first paragraph.

The defects of Masuda '400 cannot be overcome by combination with **Okuma.** The Examiner asserts that Okuma teach a method of reinserting NP cells isolated from degenerate discs. This is not the case, in fact, Okuma cautions the results he obtained using notochordal (i.e. postembryonic vestiges of the notochord/future vertebral column in **young** mammals) cells may not apply if mature NP cells are used (which is the case when cells are isolated from a **degenerate** disc, i.e. necessarily from an **adult** typically older than 4 years, which is about the age the notochord disappears in humans), compare Okuma, last sentence of the abstract, and last sentence of the discussion. Nowhere does Okuma disclose isolation from **degenerated** tissue, which poses additional challenges to healthy tissue. Embryonic/notochordal cells naturally will have a higher proliferation potential and are expected to function better than even healthy adult

tissue (without notochordal remnants) and obviously better than degenerated tissue. Notably, current claims are directed to isolation from **degenerate** disc tissue.

Unobviousness, 35 USC 103 – Masuda '473 & Libera

Masuda '473, similar to Masuda '400, does not disclose isolation from degenerate disc tissue, and culture requires fetal bovine serum, IGF-1 or other growth factors (Masuda '473, paragraph 21), as well as alginate for ECM production (paragraph 24). While the tissue source can be defective according to Masuda '473, the defects contemplated do not appear to include degeneration: "tissue which is know to be defective in some manner, **such as having a genetic defect"**. (Matsuda '473, paragraph 15). Notably, in this regard Matsuda does not contemplate degenerative tissues as source, but instead wants to use culture systems to mimic different pathological states for which "it may be desirable to isolate intervertebrate disc cells that are known to have a certain defect, such as a genetic defect" (Matsuda '473, paragraph 39). Masuda '473 teaches away from using monolayers, see paragraph 22, in particular if the cells are to be used as transplant (i.e. where phenotype and matrix formation are relevant - notably Masuda '473's embodiments are also directed to research purposes having less restrictions). As Masuda '473 points out in paragraph 32, matrix production and organization of the cells depend on culture system and growth factors and are central to transplants for surgical treatment. Furthermore, Masuda '473 tends to teach away from using cell mixtures, see Masuda '473 paragraph 13 "preferably cells are obtained from only one type of intervertebral disc source and are not mixed with intervertebral disc cells of another type, i.e. obtained NP cells are essentially

free of AF cells". Similarly, Masuda '473 points out how, if cells are to be used surgically, culture conditions can be adapted for either NP or AF tissue (implying these cells are cultured separately if used for surgery), compare Masuda '473, paragraph 33. Again, to surgically repair AF defects, AF tissue is used, and to repair NP defects, NP tissue is used, i.e. those tissues are not cultured in a mixed cell culture of AF and NP cells (Masuda '473, paragraph 37).
Notably, all of Masuda '473's examples use bovine or canine tissue and AF and NP cells were separately cultured with FBS and/or further growth factors (OP-1).

Libera does not add anything to Masuda '473, Masuda '400, and/or Okuma since Libera merely discloses a suspension culture using hydrophobic surface and a tapered bottom. However, Masuda does not teach culture of cells from degenerate disc tissue nor mixed cell cultures (AF & NP), and Libera cannot cure these defects. Furthermore, Masuda discloses the cells need to be cultured in agarose or alginate to retain their phenotype including shape and CM/ECM characteristics – this would appear to teach away from culturing them another way, in particular, in a Libera-type suspension culture. From Masuda it is unclear whether any of the various chondrogenic cells he uses would form 3D aggregates and retain sufficient proliferation to be transplanted.

None of the prior art documents, alone or in combination, disclose isolation from degenerated tissue or a mixed cell culture that allows direct cellular interaction of NP and AF cells.
To further prosecution, claim 6 has been amended include the culture of mixed NP and AF cells.

Applicant believes the claims are novel and unobvious.

Wherefore, allowance of all claims is earnestly solicited.

ADDITIONAL FEE

Please charge any insufficiency of fees, or credit any excess, to Deposit Account No. 14-1263.

Respectfully submitted,

NORRIS MCLAUGHLIN & MARCUS, P.A.

By /Gabi Klemm/
Gabi Klemm - Reg. No. 66,488
875 Third Avenue, 8th Floor
New York, New York 10022
(212) 808-0700